QUANTITATIVE ASPECTS OF THE TRANSMISSION OF *XYLELLA FASTIDIOSA* BY THE GLASSY-WINGED SHARPSHOOTER

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ABSTRACT

Transmission of *Xylella fastidiosa* (*Xf*) by the glassy-winged sharpshooter (GWSS) involves a series of events from acquisition of the bacterium to inoculation of *Xf* to a new host. While this process is often over-simplified, certain insect/pathogen interactions may be necessary to achieve a successful transmission event and the number of *Xf* cells acquired or inoculated may govern whether or not transmission will occur. In our preliminary studies, neither higher titers of *Xf* nor longer feeding periods by GWSS result in higher rates of transmission nor a greater number of bacteria transmitted.

INTRODUCTION

Solutions to PD are coming out of an understanding of basic biological aspects of the vector, the pathogen, and their hosts. The most important of these interactions is the transmission of the pathogen by the vector to a non-infected plant. The process that leads to pathogen transmission by an insect can be broken down into three separate events; (1) acquisition from an infected plant, (2) inoculation into a naive potential host, and (3) infection following inoculation. In this report, we describe the development of an artificial disease cycle for study of these relationships and have begun to describe transmission events in a quantitative fashion. Positive correlations were detected between acquisition events and total ingestion time or AAP length, but not increased number of probes. On the other end of the disease cycle, positive were detected between inoculation of *Xf* and number of probes or IAP length, but not increased total ingestion time.

OBJECTIVES

Our long-term goal is to understand quantitative aspects of the process of *Xylella fastidiosa* (*Xf*) transmission by *Homalodisca coagulata* (GWSS) in order to develop a means of reducing the efficiency with which spreads the pathogen from an infected plant to a non-infected one. Our specific objectives for this project are to:

1. Determine relationship between time a GWSS spends on a PD-infected grapevine and titer of *Xf* they acquire.
2. Determine the relationship between time a GWSS spends in post-acquisition on a non-*Xf* host and titer of *Xf* they contain.
3. Determine the relationship between time an infectious GWSS (ie, one that had acquired *Xf*) spends on a non-infected grapevine and the titer of *Xf* it inoculates into the grapevine.
4. Determine the relationship between titer of *Xf* inoculated into a plant and the probability that it will become diseased by developing a transmission index.

RESULTS

The Artificial Feeding System

We developed a simple and efficient transmission cycle for the study of *Xf* transmission by GWSS that allows detection of specific numbers of cells in plant tissue and within the insect vector by QRT PCR (3). A QRT PCR protocol for detecting the citrus variegated chlorosis strain of *Xf* has already been established (9).

QRT PCR was performed in a Rotor Gene 3000 (Corbett Research, Australia) using iQ Supermix (Bio-Rad Laboratories Inc., Hercules, CA) in 20µl reactions with *Xf*-specific 16S-23S ITS primers and the ITS probe (11). *Xf* was cultured on PD3 medium, a modification of PW medium (7,8) for 7-10 days. Bacterial cultures were scraped from a PD3 plate and suspended in sterile phosphate buffered saline (PBS). This bacterial suspension was diluted in sterile PBS to OD^{600}=2.0. Five cm sections of cut *Chrysanthemum grandiflora* stems were used for bacterial inoculations (4). The bacterial suspension was forced through the cut stem by attaching a 10cc syringe to one cut end of stem and applying pressure until the fluid was seen coming out of the other cut end. The cut ends of the stem were sealed with parafilm to prevent leakage during the acquisition access period (AAP). Five GWSS per 5 cm of stem were caged in snap cap vials for 48 h, about 250 insects placed on 50 cuttings per trial (Figure 1). Survival through the acquisition access period (AAP) indicated effective feeding because starving these insects for 48 h resulted in 100% mortality (4). After the AAP, GWSS were placed on *Xf*-free chrysanthemums for 48 h, so that any detection of bacteria would be associated with transmission and not stylet contamination.

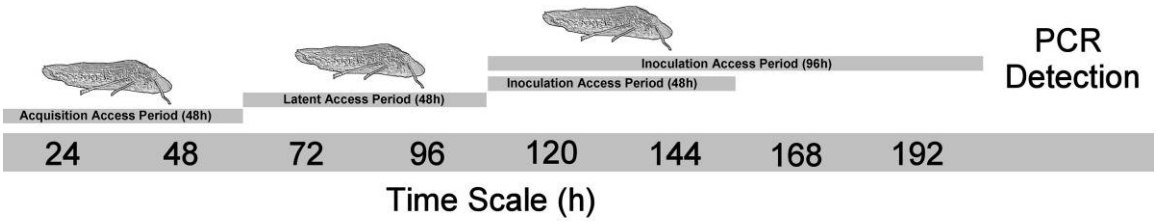

Figure 1. Artificial PD cycle for determination of *Xf* transmission.

Pairs of surviving GWSS were transferred to sterile vials containing a fresh chrysanthemum stem cutting, about 100 cuttings per trial. The insects were exposed to a stem for an inoculation access period (IAP) of 48 or 96 h. Finally, GWSS were removed from the vial and stored at 4°C until tested by PCR. DNA was extracted from the inoculation targets with the XNAR Extract-N-Amp kit (Sigma-Aldrich, St. Louis, MO) and PCR was run following a standard QRT-PCR protocol (11).

Across 9 replicates using a 48h IAP, the mean transmission rate of *Xf* by GWSS was 0.508±0.122, while the mean rate when given a 96h IAP was 0.341±0.138 (Figure 2). Using Chi-square analysis, these ratios were significantly different (X^2=16.281, df=1, p≤0.001). The lower rate associated with the longer IAP is probably due to the non-hospitable environment of the test plant stems. While the rate of *Xf* transmission was higher than previously reported (1,2,6), we feel this is a fair assessment of the insects' ability to move the bacterium from one place to another.

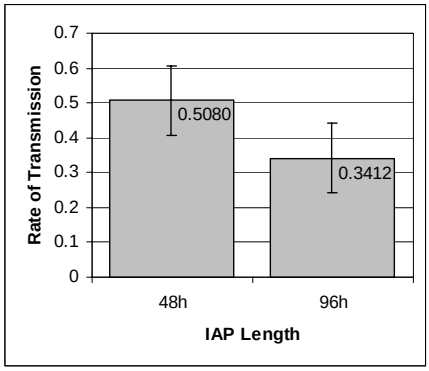

Figure 2. Rate of *Xf* transmission by the GWSS in an artificial disease cycle when given a 48 or 96 h IAP.

Distribution of Cells in the AAP Stem

In preliminary acquisition experiments using cut chrysanthemum stems with *Xf* pushed through the vascular system, great variation *Xf* cell numbers was noted, despite similar feeding times and behavior. In these experiments, 15cm cuttings were used during the "push through" portion of the process; then, stems were cut into 5cm sections and offered to GWSS. Originally, we conceptualized the push trough delivery stem as a straw that would have equally distributed cells throughout the stem. However, empirical assays determined that the stem acts more like a sieve (catching more cells at the beginning), resulting in much higher cell numbers in the first 5cm cutting (Figure 3). Therefore, we altered the "push through" step by using 5cm cuttings, without trimming the stems. We also offered GWSS a much smaller portion of the stem, affectively standardizing the access area.

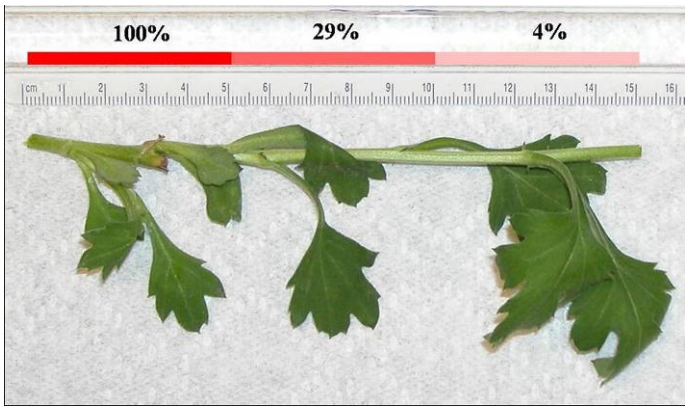

Fig 3. Distribution of *Xf* cells in 5cm sections when pushed through a 15cm cut chrysanthemum stem (n=30).

Access Period/Probe Number Correlation

GWSS were exposed to plants for 2, 4, 6, or 8 hr periods of time and monitored for two distinct feeding behaviors that could impact the transmission efficiency of the pathogen. There were strong correlations between access time and either ingestion time (r=0.97) or number of probes (r=0.76) (Figure 4**,** A and B respectively). Additionally, there was a positive correlation between number of probes and ingestion time (r=0.85).

AAP Experiments (Objective 1)

Chronologically, we started these experiments after the IAP experiments, so we have completed fewer trials, resulting in fewer data points. Despite the limited data, interesting trends have begun to surface. The ability of GWSS to acquire *Xf*

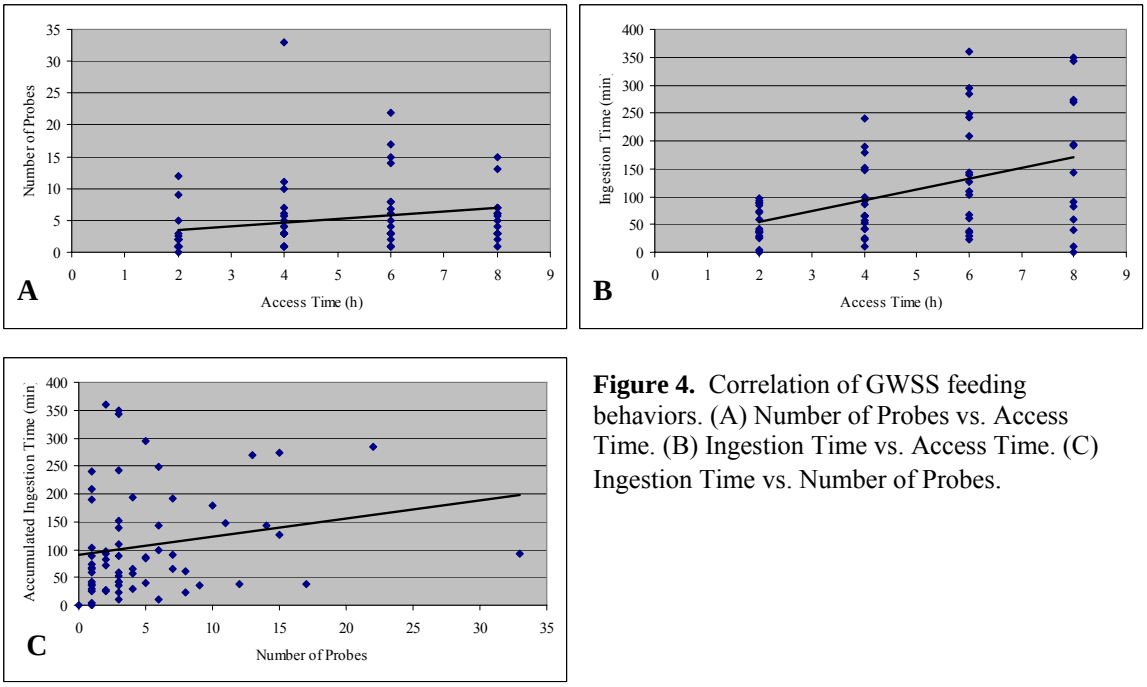

Figure 4. Correlation of GWSS feeding behaviors. (A) Number of Probes vs. Access Time. (B) Ingestion Time vs. Access Time. (C) Ingestion Time vs. Number of Probes.

from a standardized acquisition host was tested by allowing the insects to feeding on an *Xf* "push through" stem for varied periods of time (2, 4, 6, or 8 hr). During these AAP's, number of probes and total ingestion time were also recorded (Figure 5). Weak positive correlations were made between *Xf* cells in GWSS and *Xf* cells in AAP host (r=0.27), AAP length (r=0.16), and ingestion time (r=0.30). Interestingly, a negative correlation between number of probes and *Xf* cells in GWSS was made (r=-0.23). While these data are preliminary, they do follow our hypothesis that GWSS that feed longer will come in contact with more *Xf* cells. GWSS that retract their mouthparts and re-probe multiple times are less likely to ingest more *Xf* cells based on there reduced feeding time. Conversely, the more xylem fluid an insect ingests from an *Xf* infected host plant, the more *Xf* cells the insect would be expected to ingest.

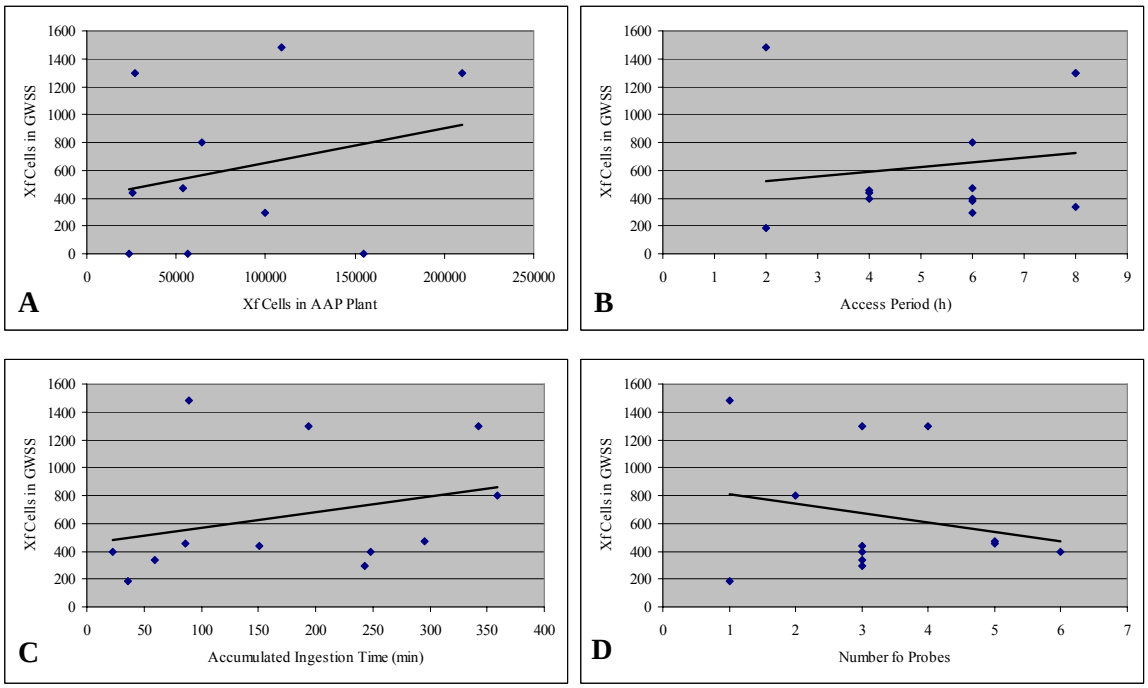

Figure 5. Number of *Xf* cells detected in GWSS by QRT PCR following feeding on a "push through" acquisition stem (Y axis in all graphs). (A) *Xf* cells vs. Number of *Xf* cells in plant. (B) *Xf* cells vs. AAP period. (C) *Xf* cells vs. Ingestion Time. (D) *Xf* cells vs. Number of Probes.

IAP Experiments (Objective 3)

The ability of GWSS to inoculate *Xf* into a target host was tested by allowing "*Xf*-positive" insects to feeding on a "clean" stem for varied periods of time (2, 4, 6, or 8 hr). During these IAP's, number of probes and total ingestion time were also recorded (Figure 6). A positive correlations was made between *Xf* cells in the target host and the length of the IAP (r=0.37). There was also a slight positive correlation between *Xf* cells in the target host and the number of probes the insects made on the target (r=0.08). This was unexpected because our hypothesis was that the more time an insect probed a host, the more cells would be transmitted. This hypothesis was based on the ingestion/egestion principle where the insect's initial contact with the xylem vessels, which are under negative pressure, would result in a backflow of foregut contents into the host. By the law of averages, the more an insect probes, the more transmission events would occur. For this reason we expected a more positive correlation. Following this line of hypothesis, we also expected a negative correlation between the total ingestion time and the number of transmitted *Xf* cells, based on the idea that active ingestion results in material moving into the GWSS foregut and not out (i.e. back into the plants xylem). Empirically, a negative correlation was made (r=-0.15), although less dramatic than we expected.

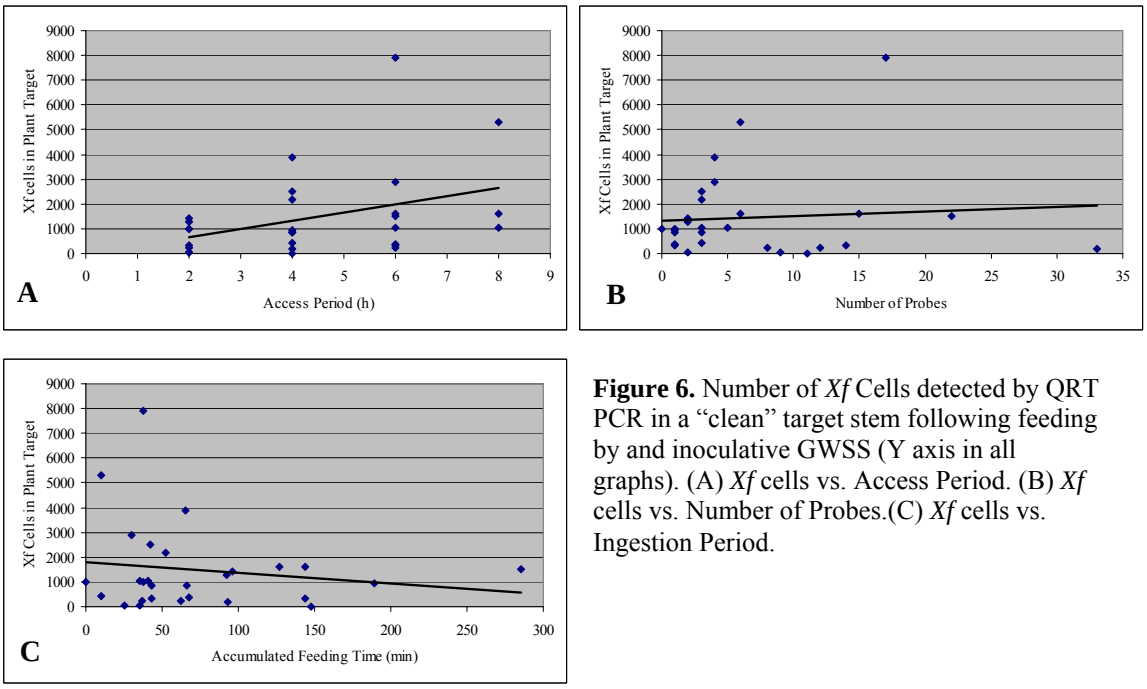

Figure 6. Number of *Xf* Cells detected by QRT PCR in a "clean" target stem following feeding by and inoculative GWSS (Y axis in all graphs). (A) *Xf* cells vs. Access Period. (B) *Xf* cells vs. Number of Probes.(C) *Xf* cells vs. Ingestion Period.

CONCLUSIONS

Movement of *Xf* from one plant to another depends on the transmission of the bacterium from an infected host to an uninfected host by the insect vector. For transmission to occur, two major events have to occur, ACQUISITION and INOCULATION. In these studies are determining behaviors and timed events that are associated with successful movement of the bacterium. Understanding these associations will allow epidemiology studies of inoculative GWSS to be more accurate and help develop a means of reducing the efficiency with which the pathogen is spread from an infected plant to a non-infected one.

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